IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Tomoyoshi ISHIKAWA *et al.*
Title:	**STABLE WATER-BASED MEDICINAL PREPARATION CONTAINING ANTIBODY**
Appl. No.:	10/584,249
371(c) Date:	06/23/2006
Examiner:	Yunsoo Kim
Art Unit:	1644
Confirmation Number:	1542

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Eiji Sawa, hereby declare as follows:

(1) I am employed by Kyowa Hakko Kirin Co., Ltd. (KHK), Japan, the assignee of the subject case, as Senior Scientist and Head of the Formulation Department of the Bio Process Research and Development Laboratories.

(2) I have been employed with KHK since 1993. I have been charged with research and development of analytical methods and formulations for antibodies since 2001.

(3) Apart from my compensation as an employee of KHK, I am not being compensated for my work in connection with the submission of this declaration. Further, my compensation is not dependent on the substance of my opinions or on the disposition of this application.

(4) I am aware that the claims pending in the subject application stand rejected in view of the combined teachings of U.S. patents No. 6,171,586 and No. 5,677,165. In particular, I understand the examiner's belief to be that it would have been obvious to

use glutamate with the antibody formulations disclosed in the '165 patent. I respectfully disagree with the examiner because, even if a researcher had considered modifying the antibody formulations of the '165 patent to include glutamate, he or she could not have predicted the superior results, discussed below, that flow from selecting a glutamate buffer over other buffers, such as citrate, that might have been considered equivalent.

(5) The formation of impurities was assessed in a first set of experiments, as a measure of formulation stability. The glutamate buffer formulation as presented in Example 5 of the application was compared to an identical formulation containing citrate in lieu of glutamate, as follows:

	"Glutamate" buffer (invention)	"Citrate" buffer (comparison)
Antibody	10 mg/ml anti-CD40 (IgG4)	10 mg/ml anti-CD40 (IgG4)
Buffering agent	10 mM sodium ***glutamate***	10 mM sodium ***citrate***
Isotonizing agent	262 mM D-sorbitol	262 mM D-sorbitol
Surfactant	0.05 mg/ml polysorbate 80	0.05 mg/ml polysorbate 80
pH	5.5	5.5

(6) Each of the formulations were stored in an incubator for 12 months at 5° C and then observed for the formation of impurities. More specifically, we sought to document the presence, if any, of microscopic particles suspended in the formulation(s).

(7) Under these conditions, we found more impurities in fact had accumulated in the "citrate" formulation than in the "glutamate" formulation, however, even after one year of storage. Because the contemporaneous literature and conventional practice had deemed glutamate and citrate buffers to have substantially identical effects in this regard, it is my opinion that the difference observed would have been surprising to those in the field.

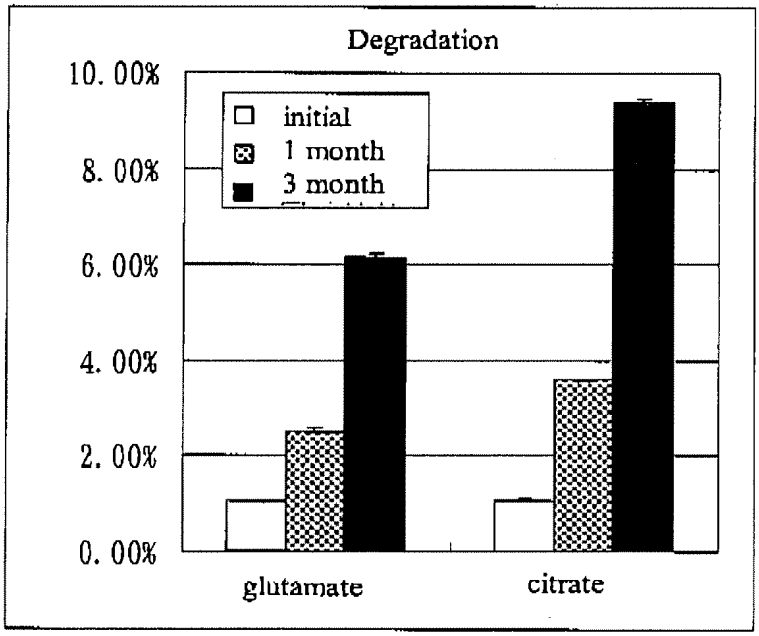
(8) Next we conducted experiments to assess the formation of impurities upon repeated freezing and thawing. Both the "glutamate" and "citrate" buffers were frozen (-20° C) and thawed (4° C) in three cycles and, after each iteration, we checked for the presence of impurities. We found more impurities in the "citrate" formulation than in

the "glutamate" formulation. Again, nothing in the contemporaneous literature and conventional practice would have prompted an expectation of this outcome.

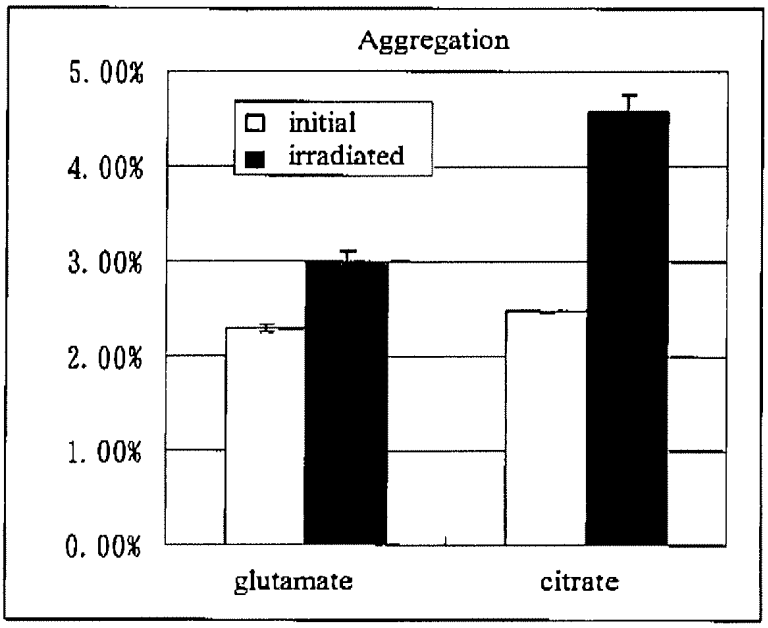
(9) As another parameter of "stability," we studied the formation of aggregates and/or the degradation of the formulated antibodies. The examined formulations differed from the aforementioned formulations only in that a IgG1 antibody was used:

	"Glutamate" buffer (invention)	"Citrate" buffer(comparison)
Antibody	10 mg/ml IgG1	10 mg/ml IgG1
Buffering agent	10 mM sodium ***glutamate***	10 mM sodium ***citrate***
Isotonizing agent	262 mM D-sorbitol	262 mM D-sorbitol
Surfactant	0.05 mg/ml polysorbate 80	0.05 mg/ml polysorbate 80
pH	5.5	5.5

(10) In a first of two experiments, both the "glutamate" and "citrate" formulations were stored in an incubator at 40° C for three months and subsequently were subjected to size-exclusion liquid chromatography after one and three months, respectively. The figure immediately below shows that the "glutamate" formulation underwent a level of degradation, compared to a "citrate" formulation, that was reduced by about 30% at both time points studied.



(11) In the second experiment, the formulations were stored under a white fluorescent lamp controlled to have approximately 4,000 lux for 300 hours and then subjected to size-exclusion liquid chromatography. As the following figure illustrates, the "glutamate" buffer of the invention reduced aggregation by about 34%, relative to a "citrate" buffer formulation.



(12) This finding is important in the field because protein aggregation can bring about immunogenicity. Whereas relatively small proteins may be "invisible" to the immune system, the same proteins upon aggregation can trigger an immune response. Thus, it is my opinion that the reduction in protein aggregation documented with the inventive "glutamate" buffers is significant to preventing an immune response and/or to reducing the severity of such response.

(13) As mentioned above, the contemporaneous literature and conventional practice had pointed to the functional equivalence of the glutamate and citrate buffers. The publications cited by the examiner, such as the '165 patent, is illustrative of this view. It is my opinion that the present application was the first to show that there can be a difference between the glutamate and other "equivalent" buffers, at least with respect to stability, and that this showing would have surprised someone with an ordinary or typical background in the field.

(14) I further declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements are so

made punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 10/28/2009 By: Eiji Sawa

Eiji Sawa